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Peptide-based imaging agents for cancer detection☆

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Abstract

Selective receptor-targeting peptide based agents have attracted considerable attention in molecular imaging of tumor cells that overexpress corresponding peptide receptors due to their unique properties such as rapid clearance from circulation as well as high affinities and specificities for their targets. The rapid growth of chemistry modification techniques has enabled the design and development of various peptide-based imaging agents with enhanced metabolic stability, favorable pharmacokinetics, improved binding affinity and selectivity, better imaging ability as well as biosafety. Among them, many radiolabeled peptides have already been translated into the clinic with impressive diagnostic accuracy and sensitivity. This review summarizes the current status in the development of peptide-based imaging agents with an emphasis on the consideration of probe design including the identification of suitable peptides, the chemical modification of probes and the criteria for clinical translation. Specific examples in clinical trials have been provided as well with respect to their diagnostic capability compared with other FDA approved imaging agents.

Keywords

Peptide; Molecular imaging; Chemical modification; Clinical experience

1. Introduction

Molecular imaging visualizes and measures biological processes at the cellular and subcellular levels within living systems. Targeted molecular imaging, which can quantify the target expression, is an indispensable tool in diagnosing and managing diseases [1–3]. A targeted imaging probe is generally composed of a targeting ligand (such as peptide, aptamer, protein or antibody), an imaging moiety (such as radioisotope for positron emission tomography (PET) or single photon emission computed tomography (SPECT), magnetic nanoparticle for magnetic resonance imaging (MRI) and organic fluorescent dye for optical

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imaging) and a linker to connect these two [4–6]. An ideal imaging probe should have high binding affinity and specificity for the particular receptor, and can be rapidly cleared from non-targets in order to ensure an adequate target-to-background ratio. In addition, high stability and integrity under physiological condition, low immunogenicity and toxicity for human exposure as well as easy production are all necessary for clinical translation.

With the help of sophisticated molecular biology, a great number of disease targets and corresponding target ligands have been discovered [7–9]. Given their unique advantages, peptides have attracted much attention for targeted imaging [10–12]. Peptides play important roles in cellular functions and intercellular communication. They are composed of amino acid monomers connected by amide bonds and typically have a low molecular weight (less than 100 amino acid residues according to the United States Food and Drug Administration (FDA) definition) which enables fast clearance from the blood as well as non-target tissue. Selected peptides generally have high affinities and specificities for their receptors and are active at concentration down to nanomolar level, therefore, resulting in desirable target-to-non-target ratios. An increasing number of peptides, such as somatostatin (SST) peptide, vasoactive intestinal peptide (VIP), Arg-Gly-Asp (RGD) peptide, and bombesin/gastrin-releasing peptide (BBN/GRP), have been successfully characterized for tumor receptor imaging [13–17].

The FDA typically handles peptides as conventional drugs instead of biological products with a focus on their compound structure [18]. Peptides normally are susceptible to chemical modification. After determining the amino acid residues for specific targeting, chemical modifications (such as cyclization, PEGylation, introduction of unnatural amino acid) are utilized to engineer the peptides for enhanced metabolic stabilities and favorable pharmacokinetics. Imaging labels are directly or indirectly conjugated to the peptides for in vivo imaging application. Accompanied by the structure modification is the possibility to lose the binding affinity and biological activity of peptides. Thus, design of chemical structure (such as insertion of appropriate linker) is explored to minimize the interaction between active binding site and unnatural modification.

In general, construction of peptide-based imaging probes involves three steps: (1) identification of the receptor and its targeting peptide; (2) design and preparation of the peptide analogs with the aim to optimize the biological activity and metabolic behavior; and (3) chemical conjugation of an imaging functionality to the peptide. Despite the great progress made in the development of peptide-based probes, their application in diagnostic imaging and monitoring therapeutic efficacy is still in its infancy. The clinical application of peptide-based agents will greatly rely on the evaluation of in vivo selectivity (whether the probe can specifically bind to its target and not to the non-target tissues); the in vivo stability (whether the probe can reach the target in an intact state); the pharmacokinetic profile (the rate and extent of the probes clears from the body) and the toxicological studies. In this review, we make a summary of the major progress in the development of peptide-based imaging agents for disease detection with a focus on demonstrating the design concept for improving the performance of imaging probes.

2. Peptides targeting receptors overexpressed in specific cancers

Targeting peptide sequences can be selected mainly in three different ways: (1) derivatization from natural proteins [19]; (2) chemical synthesis and structure-based rational engineering [20,21]; and (3) screening of peptide libraries [22]. Each method has its own strength and weakness, and is a review within itself. Among them, phage display technology is a conventional but most widely used method with many advantages such as ease of handling and large number of different peptides can be screened effectively [23].

2.1. Peptide selection/identification by phage display

Phage display technology is based on the principle of screening for specific peptides that bind to the desired target from a library of phage particles. It was introduced by George P. Smith in 1985 [24]. Since then, thousands of peptides have been screened out via phage display. In a typical in vitro phage display process, the phage surface is exposed to the foreign peptide libraries composed of small peptides varying from 5 to 45 amino acids to allow the peptides to incorporate into the phage [25,26]. Every phage clone displays one single peptide while the whole library can display up to 10^9 peptides in total. The phage display library is passed through the targeting molecules. The unbound phage is then washed off and those with desired binding activity is captured and later recovered by competitive elution. A variety of affinity selection methods have been reported to increase the chance to obtain peptides binding to the targeting molecules with good affinity. Usually, at least four rounds of iterative selection are needed to enrich phage with desired binding ability [23]. For in vivo phage display, library phages are injected into animals. Unbound phages are removed via vascular perfusion as well as additional ex vivo washing. The phages with binding activity are rescued from target organs, amplified and purified [27]. Potential candidates obtained via this identification step will be further subjected to chemical and biological evaluations for in vivo molecular imaging.

2.2. Representative peptides for in vivo imaging

The biological activities of peptides are regulated through binding with corresponding receptors. Those with receptors overexpressed on tumor cells rather than on normal cells are excellent candidates for in vivo tumor imaging. To date, many peptides and their analogs have been identified and used for disease detection (Table 1). Some representative ones are discussed below.

2.2.1. Arg-Gly-Asp (RGD) peptide—The tripeptide RGD is specifically binding to integrin receptors [60]. Integrins constitute two subunits (α and β subunits). The integrin family, especially $\alpha_V\beta_3$, is significant for tumor angiogenesis and metastasis. They are overexpressed on endothelial cells during angiogenesis, but barely detectable in most normal organs. Therefore, they are widely used for diagnostic imaging.

2.2.2. Bombesin (BBN)/gastrin-releasing peptide (GRP)—Amphibian BBNs and their related peptides consist a family of neuropeptides exhibiting various physiological effects such as exocrine and endocrine secretions, thermoregulation, sucrose regulations as well as cell growth [35]. The bombesin-like peptide receptors have 4-subtypes: the

neuromedin B receptor, the bombesin 3 receptor, the GRP receptor, and the bombesin 4 receptor. These receptors are overexpressed in many tumors such as breast cancer, ovarian cancer and gastrointestinal stromal tumors.

2.2.3. Somatostatin (SST) peptide—SSTs are naturally occurring cyclopeptide hormones with either 14 or 28 amino acids [61]. They can inhibit the secretion of insulin, glucagon and some other hormones. Their biological effects are mediated via binding to specific high-affinity somatostatin receptors (SSTRs) which are overexpressed in many tumors including but not limited to the gliomas, neuroendocrine tumors and breast tumor. Till now, five subtypes of SSTR (SSTR1–SSTR5) have been discovered.

2.2.4. Vasoactive intestinal peptide (VIP)—VIP is a neuropeptide with 28 amino acids [13]. It not only can promote vasodilation, but also can promote growth and proliferation of cells via cell-surface receptor mediated growth. Its action is mainly controlled by two receptor subtypes (VPAC1 and VPAC2). A large amount of VIP receptors are expressed on many tumors including but not limited to brain tumors, adenocarcinomas of the pancreas and neuroendocrine tumors. It is worth mentioning that VIP is also a potential drug and doses at submicrogram level can cause toxic effect.

2.2.5. Cholecystokinin (CCK)/gastrin peptide—CCK and gastrin are structurally and functionally similar peptides that exert a variety of physiological actions in the gastrointestinal tract as well as the central nervous system [62]. They have an identical sequence at the biologically active part but differ in sulfation position of the tyrosine (position 6 for gastrin and position 7 for CCK). Till now, three types of receptors for CCK (CCK1, CCK2 and CCK2i4sv) have been identified, which all belong to the superfamily of GPCRs. Among them, CCK2/gastrin receptors have been frequently found in human cancers such as stromal ovarian cancers and astrocytomas.

2.2.6. α-Melanocyte-stimulating hormone (α-MSH)—α-MSHs are linear tridecapeptides, mainly responsible for skin pigmentation regulation [63]. α-MSHs and their analogs exhibit impressive binding affinities to melanocortin-1 receptors (MC-1r) which are expressed in over 80% of human melanoma metastases, and thus, are widely used as vehicles for melanoma-targeted imaging and radiotherapy.

2.2.7. Neutrotensin (NT)—NT is a peptide with 13 amino acids, targeting NT receptor which has been identified in various tumors such as ductal pancreatic adenocarcinomas, small cell lung cancer, and medullary thyroid cancer [64]. Therefore, it is an attractive candidate for cancer imaging.

2.2.8. T140—T140, a peptide with 14 amino acids and one disulfide bridge, is an inverse agonist of chemokine receptor type 4 (CXCR4) [65]. Its derivatives are widely used as CXCR4 imaging agents.

2.2.9. Exendin-4—Exendin-4 is a 39-amino acid peptide hormone [66]. It has 50% homology in the amino acid composition with native glucagon-like peptide 1 (GLP-1) and is

found to be an agonist for GLP-1 receptor. Exendin-4 has been clinically used for the treatment of patients with insulinoma and type 2 diabetes.

2.2.10. Neuropeptide Y (NPY)—NPY is a peptide with 36 amino acids and belongs to the pancreatic polypeptide family [67]. NPY receptors are overexpressed in various tumors including neuroblastomas, sarcomas, and breast cancers. Many NPY analogs have been evaluated in animal models.

2.2.11. Substance P—Substance P is an undecapeptide belonging to a family of neuropeptides known as tachykinins [68]. Substance P is a specific endogenous ligand known for neurokinin 1 receptor (NK_1R) which is found to be expressed on various cancer cells. Substance P analogs are synthesized and used for NK_1R positive tumor detection.

2.2.12. Tumor molecular targeted peptide 1 (TMTP1)—TMTP1 is a 5-amino acid peptide that has been found to specifically bind to highly metastatic cancer cells, especially those from a typical liver micrometastasis [69]. However, high concentration of TMTP1 could mediate tumor cell apoptosis. Therefore, suitably labeled TMTP1 has been tested pre-clinically for imaging and therapeutic applications.

2.3. Agonists vs. antagonists

Distinguishing the concept of “antagonist” from “agonist” is vital for the design of peptide based imaging probes. Most of the peptides mentioned above could cause physiological changes with receptor binding and are called “agonists”. Conversely, the peptides that bind to the receptors without activation changes are termed “antagonists”. It was originally believed that receptor agonists will be internalized and retained in the cell, therefore, more appropriate than antagonists for imaging. However, more and more studies have showed the advantages of antagonist for in vivo imaging. For example, Ginj et al. developed a radiolabeled SSTR2 antagonist (^{111}In-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA)-[4-NO_2-Phe-c(DCys-Tyr-DTrp-Lys-Thr-Cys)-DTyr-NH_2]) and compared the in vivo tumor targeting properties with a highly potent radiolabeled SSTR2 agonist (^{111}Indiethylenetriaminepentaacetic acid (DTPA)–[Tyr^3,Thr^8]-octreotide) [45]. Although the antagonist showed a lower cellular receptor affinity than that of the agonist, the in vivo tumor retention of the antagonist is nearly twice that of the agonist. In another study, Cescato et al. compared the in vitro and in vivo tumor targeting properties of a bombesin agonist (N_4-[Pro^1, Tyr^4, Nle^{14}]Bombesin) with a bombesin antagonist (N_4-[D-Phe^6, Leu-$NHEt^{13}$, des-Met^{14}]Bombesin) [34]. Compared with the agonist, the antagonist exhibits similar binding affinity, no active internalization and a three-fold higher tumor uptake. They ascribed this enhanced tumor uptake of antagonist to its binding ability to more receptor sites, slower dissociation rate from the receptor as well as its stronger resistance to membrane-bound enzymes. Besides, since the antagonists exhibit much less side-effects than agonists, they are more clinically preferred.

3. Chemical modification of a peptide

Despite their specific targeting ability and desirable pharmacokinetics, native peptides are seldom directly used for in vivo imaging. Peptides usually have a very short in vivo

biological half-life of around several minutes. Suffering from enzymatic degradation as well as fast renal clearance, they may lose their bioactivity even before reaching the intended target.

However, peptides are susceptible to chemical modification which can greatly enhance their proteolytic stability, improve their water solubility and reduce their renal clearance [70,71]. The strategies to develop metabolically stable peptides include cyclization, the use of more stable amino acids, modification of peptide C- and N-termini and the use of pseudo-peptide. To prevent the peptides from fast renal clearance, synthetic strategies like polymer fusion and attachment to long-lived proteins are explored. Furthermore, the synthetic strategies of dimers, tetramers and heterodimers are employed to increase the receptor affinity of monomeric peptides.

3.1. Strategies to enhance the metabolic stability of peptides

Cyclization often increases the metabolic stability of peptides [72]. The prolonged biological activity is mainly due to two reasons: first, cyclization can help avoid degradation by reducing the formation of conformers susceptible to proteolytic enzymes [73]. Second, cyclization protects the N- or C-terminus of peptide sequence which is the cleavage site of exoproteases [74]. Bogdanowich-Knipp et al. showed that the cyclic RGD peptide (cyclo-(1,6)-Ac-CRGDF-Pen-NH_2) was much more stable than the linear one at biological pH [75]. Their hypothesis was that cyclization of RGD peptides via disulfide bond can induce structural rigidity and prevent the degradation regulated by the aspartic acid residue. The ring size of the cyclic peptide also matters. Octreotide is a clinically used SST like peptide drug. Compared with native SST-14, octreotide has used more stable D-Trp for L-Trp and the ring size has been decreased from 12 to 6 [76]. When maintaining the receptor binding affinity of somatostatin, octreotide has a prolonged plasma half-life of about 100 min in humans.

Incorporation of more stable amino acids, such as D-amino acids [77] and β-Ala [78], is also widely used to improve enzymatic stability [77]. Substituting D-tyrosine by D-phenylalanine in cyclo(RGDyK) results in cyclo(RGDfK). Compared with cyclo(RGDyK), cyclo(RGDfK) is more stable during various treatments such as heating, pH adjustment and serum incubation. This greatly benefits development of RGD based imaging probes which may require heating and may cause oxidation of the tyrosine but not phenylalanine.

Modification of a peptide bond to obtain non-peptidic structure which is not cleavable by peptidases, is another way to alter the physicochemical characteristics of a peptide [70]. Jenson's group has reduced the CO-NH linkage of bombesin to CH_2-NH during the solid-phase synthesis. This approach highly improved the resistance towards enzymatic hydrolysis in the modified position. What's more, the [Leu^{14}-ψ-CH_2NH-Leu^{13}]Bombesin they synthesized exhibited a 100-fold improvement in binding affinity than the corresponding bombesin receptor antagonists [79]. However, not all the bond modifications can maintain or improve the biological activity. Elimination of a peptide bond CO group probably causes the loss of potential intramolecular hydrogen bonding point and increases the rotation of C—N bond.

3.2. Strategies to prolong the blood circulation of peptides

PEGylation can improve the pharmacokinetic properties of peptides significantly by increasing their water solubility and reducing their reticuloendothelial system (RES) or renal clearance [71]. One or multiple polyethyleneglycol (PEG) chains with molecular weight ranging from several hundreds to several thousands have been attached to different peptides. The PEGylated dimeric RGD has a much lower liver uptake around 2.25 ± 0.26%ID/g compared with 4.38 ± 0.39%ID/g of dimeric RGD [80]. In another study, linkage of a 10 kDa PEG to HM-3 (an RGD modified endostatin-derived synthetic peptide) elongates its half-life in the male SD rats by a factor of 5.86 (from 27.66 ± 7.37 min to 162.08 ± 36.57 min) [81]. Glycosylation is also investigated to increase the hydrophilicity of peptides or peptide derivatives, and thus decrease their hepatic accumulation. [^{18}F]Galacto-RGD, designed by attaching a sugar amino acid to the cyclic peptide c(RGDfK), showed a liver uptake of around 0.8%ID/g in U87MG tumor bearing mice 60 min post-injection which is much less than that of corresponding RGD (around 1.6%ID/g) [82]. Human serum albumin, a 65 kDa protein that is abundant in the circulation system with a half-life of around 20 days, has been used to improve the blood circulation of peptides as well. Chen et al. have covalently conjugated IRDye800 (organic fluorescent dye) and RGD peptide with human serum albumin. This probe exhibits a prolonged circulation half-life and has been successfully used for tumor imaging [83]. An alternative to the direct attachment with albumin is to use the albumin-binding molecules. Albumin-binding molecules, such as phenol red, fatty acid and Evans blue (EB) dye, can indirectly tether the peptides to the long-lived serum protein [84]. Recently, Chen's group conjugated truncated EB (tEB) to exendin-4 peptide, resulting in an albumin binding drug candidate, denoted as Abextide [85]. The tEB conjugation did not comprise the binding affinity of exendin-4, however, greatly increased its half-life from 5.16 ± 5.23 h to 36.28 ± 7.01 h.

3.3. Strategies to increase the binding affinity of peptides

The introduction of the dimeric or multimeric peptide system is expected to enhance receptor targeting. Although specific targeting ability of each individual peptide is weak, through cooperative interaction, the overall binding affinity of multimeric system is expected to be greatly enhanced. Janssen et al. proved that the ^{99m}Tc modified RGDfK dimer (^{99m}Tc-HYNIC-E-[c(RGDfK)]$_2$) has 10 times higher binding affinity than ^{99m}Tc modified RGDfK monomer (^{99m}Tc-HYNIC-c(RGDfK)) [86]. Wu et al. further demonstrated that the binding avidity of RGDfK tetramer is two times higher than RGDfK dimer [87]. The ^{64}Cu labeled tetrameric RGD peptide appears to be an excellent ligand for in vivo integrin targeting with a rapid and high U87MG tumor accumulation around 9.93 ± 1.05%ID/g at 30 min.

To optimize the polyvalent effect of multimeric peptide, a large variety of linkers are incorporated between the peptides and the imaging moieties, the length, flexibility and hydrophilicity of which all need to be considered. Guo et al. synthesized three ^{18}F labeled dimeric RGD peptides: ^{18}F-AlF-NOTA-E[c(RGDfK)]$_2$ without PEGylation, ^{18}F-AlF-NOTA-PEG$_4$-E[c(RGDfK)]$_2$ with a PEG$_4$ linker between radio-chelator NOTA (1,4,7-triazacyclononane-1,4,7-triacetic acid) and RGD dimer, and ^{18}F-AlF-NOTA-E[PEG$_4$-c(RGDfk)]$_2$ with PEG$_4$ linkers between two RGDfK [88]. It is understandable that radiochelator somewhat reduces the receptor binding of RGDfK and the PEGylation will

restore the binding affinity to some extent. The existence of two PEG_4 linkers further provides a suitable distance between two RGDfK for simultaneous binding in a bivalent fashion. Thus, the binding affinity of these peptides followed the order of NOTA-E[PEG_4-c(RGDfk)]$_2$ > NOTA-PEG_4-E[c(RGDfK)]$_2$ > NOTA-E[c(RGDfK)]$_2$. The ^{18}F-AlF-NOTA-E[PEG_4-c(RGDfK)]$_2$ shows its superior capability for in vivo integrin $\alpha_v\beta_3$ imaging with relatively low liver accumulation and high tumor uptake. Liu et al. found that by increasing the length of linker between two cyclic RGD motifs, the binding affinity of RGD dimer was highly improved [89]. They inserted the Gly_3 or PEG_4 linkers between each RGD. The distances between two cyclic RGD motifs are 6 bonds in NOTA-E[c(RGDfK)]$_2$, 26 bonds in NOTA-E[Gly_3-c(RGDfK)$_2$] and 38 bonds in NOTA-E[PEG_4-c(RGDfK)$_2$]. Although no direct evidence to prove the simultaneous binding of two RGD motifs after inserting the linkers, the binding affinity of NOTA-E[Gly_3-c(RGDfK)$_2$] and NOTA-E[PEG_4-c(RGDfK)$_2$] is significantly increased compared with NOTA-E[c(RGDfK)]$_2$. In vivo tumor uptake further confirmed the increased binding affinity with tumor uptake around 9.04 ± 2.05%ID/g, 10.13 ± 1.81%ID/g and 5.28 ± 1.03%ID/g for ^{68}Ga-NOTA-E[Gly_3-c(RGDfK)$_2$], ^{68}Ga-NOTA-E[PEG_4-c(RGDfK)$_2$] and ^{68}Ga-NOTA-E[c(RGDfK)]$_2$ at 30 min post-injection respectively in the integrin $\alpha_v\beta_3$-positive U87MG tumor model.

Besides using the same type of peptide ligands to construct homodimers or homomultimers, different types of peptide ligands can be put together with suitable linkers to form heterodimers for targeting multi-receptor over-expressed tumor cells. The understanding of receptor expression pattern on cells is of great importance for the design of heterodimer with high affinity and specificity. As androgen-independent prostate cancer cells overexpress both gastrin-releasing peptide receptor (GRPR) and integrin $\alpha_v\beta_3$, radiolabeled BBN-RGD peptide heterodimers with BBN motif for GRPR targeting and RGD motif for integrin targeting have been developed to improve the imaging results over both radiolabeled BBN and RGD monopeptide. Liu et al. linked bombesin and RGD via a glutamate linker and further conjugated NOTA for ^{64}Cu radiolabeling [90]. Due to the synergistic effect, the ^{64}Cu-NOTA-RGD-BBN showed a much higher tumor uptake than ^{64}Cu-NOTA-RGD, ^{64}Cu-NOTA-BBN and even ^{64}Cu-NOTA-RGD plus ^{64}Cu-NOTA-BBN in PC-3 tumor at 4 h post-injection. The in vivo integrin and GRPR dual-receptor binding ability were further proved by the in vivo blocking studies. The tumor uptake of these probes can be totally blocked with both RGD and bombesin. Either RGD or bombesin will only partially inhibit the tumor uptake. In a later study, they modified the heterodimer by inserting a PEG_3 spacer, 11-amino-3,6,9-trioxaundecanoic acid, onto the glutamate group and labeled it with ^{18}F-FB (^{18}F-fluorobenzoyl) prosthetic labeling group [91]. The PEG_3 greatly increased the hydrophilicity of the heterodimer, leading to a faster renal clearance and more favorable pharmacokinetic behavior. Other heterodimers have been reported including but not limited to MSH/CCK [92], RGD/octreotate [93], and RGD/ATWLPPR [94].

The linkers, either rigid or flexible, between the two different peptides are of great concern for heterodimer since it has to present ligands simultaneously to the receptors with the lowest entropy and optimized conformation. Vagner et al. used short flexible ethylene glycol (PEG) spacers together with semirigid Pro-Gly (PG) repeats as linkers to connect MSH and Delt-II (Deltorphin-II) binding moieties [95]. Among 8 different linkers, -PG_n- (n = 3, 6, 9, 12, 15) and -PEG–[PG]$_m$–PEG-(m = 6, 12, 18) with different lengths from 13 to 96 Å, the

cell binding experiment proved that the optimal linker length to span both receptors (melanocortin-4 for MSH and δ-opioid for Delt-II) is around 20–50 Å. The result is consistent with the modeling study.

It is worth mentioning that chemical modification also could improve the binding selectivity of peptides. For example, cyclization could constrain the geometries of linear peptides to certain isoforms for targeted receptors. Pfaff et al. found that compared with linear GRGDS, the cyclic pentapeptide (RGDFV) was 10-fold more active to $\alpha_v\beta_3$ but equal in activity to $\alpha_5\beta_1$ [96] (Fig. 1).

4. Imaging functionality

4.1. Traditional imaging functionality

The selected peptides and peptide analogs need to be labeled with imaging moieties in order to serve as in vivo imaging probes. Some widely used imaging moieties include organic dyes for optical imaging, radionuclides for PET and SPECT imaging and Gd^{3+} chelators for MR imaging [11]. The functional groups of peptides available for conjugation include but are not limited to the ε-amino group on lysine side chains, the guanidinium group on arginine side chains, the carboxyl groups on aspartic acid or glutamic acid, the cysteine thiol, and the phenol on tyrosine [99]. The most common conjugation reactions are carbodiimide/*N*-hydroxysuccinimidyl (EDC/NHS) mediated carboxyl and amine coupling, maleimide conjugation to thiol groups, and diazonium modification of the phenol on tyrosine. Fig. 2 highlights the representative chemistries to couple peptides with imaging moieties. Details can be found in a good number of reviews [100,101].

Radiolabeled peptides are the most widely used peptide-based imaging agents [102]. Radionuclide based PET/SPECT imaging could detect the imaging agents at micromolar to picomolar concentration. Therefore, it is possible to minimize the usage of peptides in order to reduce any adverse biological effect. To develop peptide based probes for PET/SPECT imaging, radionuclides should be labeled onto targeting peptides. Several radionuclides employed for peptide labeling are ^{99m}Tc, ^{123}I, and ^{111}In for SPECT imaging and ^{18}F, ^{64}Cu and ^{68}Ga for PET imaging [103]. Generally, these radionuclides are attached to the peptides via chelators or post-synthetic radiolabeling groups. Some widely-used chelators are listed in Fig. 3.

The radiochemical stability is of critical importance for PET/SPECT imaging probe development [104]. The choice of radionuclides is very significant for peptides: due to their small size, the receptor binding affinity and pharmacokinetic patterns can be strongly affected with the attachment of different radionuclides. Liu et al. labeled RGD-BBN with three different radioisotopes: ^{68}Ga and ^{64}Cu via NOTA chelation and ^{18}F via FB prosthetic labeling group [105]. The metal isotopes (^{68}Ga, ^{64}Cu) labeled RGD-BBN showed higher tumor uptake than ^{18}F labeled RGD-BBN in both T47D (GRPR+/integrin $\alpha_v\beta_3$ −) and MDA-MB-435 (GRPR−/integrin $\alpha_v\beta_3$ +) breast cancer models while the ^{18}F labeled RGD-BBN showed a faster wash out than the former two. The radiolabeling site also plays an important role to maintain the biological activity of the peptides. It is necessary to understand the active sequence of peptides so that the radiolabeling can be directed at a site

away from the receptor binding site. Usually, the sequence conferring the binding affinity is at the carboxy terminus while the chelator is modified to the amino terminal group. If necessary, spacers like PEG are used to further extend the distance between the receptor binding site and chelation site [106]. Specific examples have been discussed in Section 3.3.

The design of fluorophore labeled peptides is similar to radiolabeled peptides except that fluorophores are used to replace radionuclides. Various dyes are commercially available (for examples, Cyanine dyes from GE Healthcare and Alexa Fluor dyes from Invitrogen). Cheng et al. have conjugated Cy5.5 to RGD monomer, dimer and tetramer for in vivo optical imaging [107]. The binding affinities showed the same trend as those for radiolabeled RGD. The Cy5.5 labeled RGD tetramer showed the highest tumor-to-normal tissue ratio at 4 h post-injection. Although the fluorescence imaging has the advantages of high resolution, non-invasive and safe detection, the use of fluorophores in vivo is limited by the light penetration and tissue autofluorescence. The introduction of hybrid derivatives containing both a fluorescent tag and a radioactive label opens a new avenue to the field of image-guided surgery. Zhu et al. have conjugated cyclic RGD peptide with both DOTA for ^{64}Cu labeling and a near-infrared ZW-1 dye [83]. The c(RGDyK) peptides did not lose their biological activity after dual-labeling. The tumor region in preclinical xenograft models can be clearly seen via both optical imaging and PET imaging with high tumor-to-background contrast. In another example, by taking the advantage of the strong interaction between streptavidin (SAv) and biotin, Kang et al. conjugated ^{64}Cu-labeled Alexa Fluor 680-streptavidin with biotin-PEG-dimeric cyclic RGD peptide [108]. IC_{50} value of this probe (DOTA-(AF)SAv/biotin-PEG-RGD_2) was 3.1-fold higher than that of RGD_2. This imaging agent provided specific and sensitive PET/optical dual modality images for monitoring integrin $\alpha_V\beta_3$ expression. For the design of peptide-based probes for multimodality imaging, a major concern is that the relatively large size and complicated structure of the tags may strongly affect the original binding affinity and biological activity of peptides.

4.2. Nanoplatform based imaging functionality

The emerging nanotechnology provides new platforms for peptides [109–112]. Nanomaterials with at least one dimension within 1 to 100 nm have large surface areas allowing the efficient modification of multiple targeting peptides as well as imaging modalities. This could greatly improve the binding affinity of single peptide via a polyvalent effect. Further, nanoparticles themselves have unique physicochemical properties for theranostic applications. For example, semiconducting quantum dots (QDs) have higher fluorescence quantum yield and better photostability than traditional fluorescent dyes for optical imaging; superparamagnetic iron oxide nanoparticles are FDA approved MRI agents. Furthermore, the blood half-lives of nanomaterials are highly dependent on their size, shape and surface modifications [113,114]. By engineering the nanoplatforms, especially the surface coating, it is possible to improve the blood half-life and targeting efficiency of peptides.

Generally, peptides are attached to the nanomaterials via three strategies: covalent conjugation, electrostatic assembly, and selective noncovalent binding interactions (Fig. 4) [111]. Covalent conjugation can provide a rigid attachment. The conjugation chemistries for

nanomaterials are similar to those for small molecules. Details have been discussed in Section 4.1. Cai et al. conjugated SH-c(RGDyK) peptide to poly(ethylene glycol) coated quantum dots via maleimide-thiol coupling [115]. Each QD was estimated to be coated with around 90 copies of c(RGDyk). Meanwhile, the QD-RGD conjugate was coupled with DOTA for ^{64}Cu labeling. These DOTA-QD-RGD nanoparticles exhibited 60-fold-higher integrin $\alpha_V\beta_3$ avidity than c(RGDyK). The in vivo dual PET/NIRF imaging was successfully obtained in mice bearing U87MG tumor (Fig. 5). In a similar way, Lee et al. have conjugated polyaspartic acid coated iron oxide nanoparticles with DOTA and SH-c(RGDyK) for PET/MRI dual modality tumor imaging [116]. Liu et al. have modified single-walled carbon nanotubes (SWNTs) with PEGylated phospholipids and further conjugated with c(RGDyK) for integrin targeting and DOTA for ^{64}Cu labeling [117]. In both cases, the tumor uptake of nanoparticles with active targeting RGD peptides was much higher than those without RGD modification. Although conjugation of different peptides to the surface of nanoparticles provides an effective way to develop target-specific multimodal imaging agents, the formation of chemical bonds may interfere with the targeting ability [106,118].

Electrostatic attachment is the simplest method for NP bioconjugation by making use of the attraction between the charged nanomaterials and the oppositely charged peptides. Wagner et al. used positively charged peptide VW05 (H_2N-Abz-LERKLKELERKLKELERKLKELERKL-COOH) as a template for the assembly of negative charged 11-mercaptoundecanoic acid modified Au nanoparticles [119]. Since the nanoparticle–peptide interaction is dependent on electrostatic forces, the assembly and disassembly of colloids and peptides can be controlled by pH of the solution. However, the concerns that the peptide can be easily dissociated from the complex due to the interruption of the pH and ionic strength hinder the application of this kind of probes for in vivo imaging.

An alternative strategy to maintain the bioactivity of peptides without sacrificing the rigid interaction between peptides and nanocomplex is to utilize some specific noncovalent binding between peptides and nanomaterials. A representative example is to form stable coordination complexes between the electron-donating amino acids, especially histidine, and the transition metals such as Ni(II) and Zn(II). Choi et al. used a peptide which consists of a triplicate tandem repeat of ZnO binding motif (RPHRK) and flexible linker (GGDA) to modify Fe_3O_4/ZnO core/shell nanoparticles [120]. The binding constant of this peptide to ZnO surface is around 1.4×10^6 M^{-1}. Fused with this peptide, an over 10 times CEA (a tumor antigen) loading was achieved with the same amount of nanoparticles. This nanocomplex provides a simple way for ex vivo antigen loading of dendritic cells and for in vivo tracking. Bioconjugation of Au or semiconductor NPs with thiolated peptides is another typical example of dative bonds: the sulfur atom of thiol can contribute a lone pair of electrons to the empty orbital of gold atom or semiconductor atom at the interface. Pinaud et al. have synthesized a phytochelatin-related peptide as an organic coating for CdSe/ZnS core/shell nanoparticles [121]. The peptide was designed to directly bind to the nanocrystals via multiple repeats of cysteine pairs. A flexible hydrophilic linker was followed to promote the colloidal stability. Shi et al. have modified the CdSe/ZnS QDs with tetrapeptide RGDC and further labeled the peptide molecules with rhodamine dyes as energy acceptor [122]. Due to the fluorescence resonance energy transfer (FRET) between the QDs and the

attached rhodamine molecules, the emission color of QDs changed from green to orange. Cleavage of the peptide by extracellular matrix metalloproteinases (MMPs) resulted in a decrease in FRET efficiency and the recovery of the green emission. This design enables monitoring the activity of specific proteolytric enzymes. This selective non-covalent binding strategy in general is highly dependent on the discovery of peptidyl sequences which can specifically bind to NP with high affinity. However, although stronger than electrostatic adsorption, dative bonds still face the challenges of pH change, oxidation as well as competition from other similar biomolecules.

Recent development in biotechnology and nanotechnology has offered various exciting possibilities for the development of multifunctional nanoparticles to target-specific delivery of theranostic agents [110,123,124]. To construct nanoplatforms with peptides as targeting agents, the unique properties of peptides (such as the low molecular weight and relative metabolic instability) place extra criteria for optimal construction: (1) minimizing the resulting hydrodynamic size of nanocomplex; (2) controlling the peptide display orientation and peptide separation distance; (3) avoiding access to non-natural residues and limiting chemical diversity during preparation; and (4) demanding purification before administration [109]. Some construction strategies for other purposes like encapsulating peptides inside hollow or mesoporous nanomatrix for therapeutic application are referred to other reviews [125,126].

5. Clinical application

An imaging probe suitable for clinical use must satisfy the following criteria: it must dominantly accumulate in the target tissue but not in the normal tissues; it should be stable enough to reach its target in an intact state but also can be fast cleared from the circulation to minimize the background and to avoid long-term exposure; all the toxicological studies, pharmacokinetics studies and preclinical tests must be passed. Due to the chemical complication and unknown toxicology, till now, very few peptide modified nanoplatforms have been translated into the clinic, although some nanocomplexes do show impressive tumor targeting and imaging capacity preclinically. Radiolabeled peptides have been applied in a wide range of hospitals continuously with their favorable pharmacokinetic behavior.

To date, several tens of radiolabeled peptides have been developed and clinically used for diagnosis and therapy. For example, ^{111}In-DTPA-octreotide (OctreoScan; Mallinckrodt Inc.) has been used as the gold standard for the diagnosis and staging of SSTR positive tumors since the 1980s [127]. ^{99m}Tc-RP527, a bombesin analog based probe, has shown specific tumor localization and excellent imaging characteristics in humans with prostate and breast cancer [128]. ^{111}In-labeled derivatives of the insulinotropic 39-mer peptide exendin-4 were proven to be beneficial in both pre- and intraoperative localization of benign lesions [129].

Peptides have been chemically engineered with the aim for enhanced metabolic stabilities and more favorable pharmacokinetics. Take RGD peptide as an example, radiolabeled derivatives which have been used in the clinical trials include but not limited to ^{99m}Tc-αP2, [^{18}F]Galacto-RGD, [^{18}F]Fluciclatide (also known as [^{18}F]AH111585), [^{18}F]RGD-K5, ^{18}F-FPPRGD$_2$, [^{18}F]Alfatide, [^{68}Ga]NOTA-PRGD$_2$, and ^{99m}Tc-3PRGD$_2$ [130]. In 1998,

Sivolapenko et al. first reported the results of fourteen patients diagnosed with metastatic melanoma using ^{99m}Tc-αP2, which is a linear decapeptide with two RGD motifs [131]. Due to the fast removal of synthetic peptide from the circulation, the imaging quality is not satisfactory. Various chemical modifications, such as cyclization, glucolysation, PEGylation and multimerization, have been made to optimize the imaging quality. [^{18}F]Galacto-RGD, which contains one cyclic RGD motif and a glucosamine moiety, was the first RGD based PET tracer tested in human [132,133]. The biodistribution of [-^{18}F]Galacto-RGD demonstrated specific receptor binding and rapid renal clearance in cancer patients. [^{18}F]Fluciclatide, a compound optimized by the cyclization, PEGylation and introduction of two disulfide bonds, was developed by GE Healthcare. Kenny et al. reported that the phase I trial of [^{18}F]Fluciclatide in breast cancer patients with all 18 tumors was visible on the PET images and confirmed by CT [134]. [-^{18}F]FPPRGD$_2$, a PEGylated dimeric RGD peptide, has been developed and approved by the FDA as the first dimeric RGD peptide for human trial [135]. It has stable kinetics for imaging integrin $\alpha_v\beta_3$ expression, feasible early assessment of response to anti-angiogenic treatment and demonstrates encouraging results in patients with glioblastoma multiforme (GBM).

A simple, automatic, high-yield synthetic procedure is of great help to clinical translation. Especially for procedures involving radioisotopes, saving time is important due to their decay characteristics. Radiolabeling of [^{18}F]Galacto-RGD requires coupling of 4-nitrophenyl-2-[^{18}F]fluoropropionate to precursor of glycopeptide. The whole process is around 200 min, involving 4 steps of radiosynthesis and 3 rounds of HPLC purification [132]. Thus, [^{18}F]RGD-K5, a compound having a similar structure with that of [^{18}F]Galacto-RGD but suitable for a simplified radiofluorination method via click chemistry, was developed [136]. The reaction time was decreased to 90 min with the possibility for (semi)automated synthesis. Since direct labeling of ^{18}F suffers from the problems like multistep and time-consuming, [^{18}F]fluoride-aluminum complexes have been developed for peptide labeling. Chen's group have successfully synthesized [^{18}F]AlF-NOTA-PRGD$_2$ (donated as [^{18}F]Alfatide) [137] and subsequently, a more stable tracer [^{18}F]NOTA-E[PEG$_4$-c(RGDfk)]$_2$ (denoted as [^{18}F]Alfatide II) [138]. ^{18}F-fluoride strongly binds to the aluminum which is chelated by NOTA chelator. The whole labeling process can be accomplished within 40 min. They further simplified the reagents into lyophilized kit formulation and reduced the reaction time to 20 min including cartridge purification for wide-spread use in the clinic.

The possibility to obtain the isotopes (either from its own radiochemistry facility or transport from other centers) is another barrier for translating radiolabeled peptides to the clinical setting. Isotopes have been widely used in clinical trials including ^{18}F and ^{68}Ga for PET imaging, ^{99m}Tc and ^{111}In for SPECT imaging and ^{90}Y and ^{177}Lu for radiotherapy. ^{111}In-DTPA-octreotide serves as an effective prognostic standard for well-differentiated malignant endocrine tumors [139]. However, ^{111}In is not ideal for clinical use due to its unfavorable nuclear physical properties, and limited availability. ^{99m}Tc labeled somatostatin analogs, such as ^{99m}Tc labeled depreotide (^{99m}Tc-NeoTect, Diatide, Inc.) [140] and ^{99m}Tc-N4-[Tyr^3]octreotate [141], become more and more important because of their better imaging quality and the wide availability of ^{99m}Tc. Compared with SPECT imaging, PET imaging with ^{68}Ga and ^{18}F labeled somatostatin analogs has higher sensitivity and reduced scanning

time for neuroendocrine tumor (NET) detection. Somatostatin peptides have been radiolabeled with ^{18}F and ^{68}Ga via different chemical methods [142–145]. ^{68}Ga labeled tracers have certain advantages over ^{18}F labeled ones since the generator based ^{68}Ga production makes it possible to prepare compounds easily even in centers without an on-site cyclotron. ^{68}Ga labeled DOTA conjugated [Tyr^3]octreotide (TOC) [142], [Tyr^3, Thr^8]octreotide (TATE) [146], and [1-Nal^3]octreotide (NOC) [147] are the most popular ^{68}Ga-labeled somatostatin analogs clinically used for NETs. A great number of studies reported a higher tumor-to-tissue contrast and a higher sensitivity for PET/CT detection compared with CT and somatostatin receptor scintigraphy (SRS). Although this review focuses on the imaging application, it is worth to note that peptides are also excellent candidates for peptide receptor targeted radiotherapy (PRTR) due to their fast clearance ability and specific binding ability which leads to minimal exposure of health tissues [14].

Many radiolabeled peptides exhibit diagnostic advantages over other imaging procedures in clinical trials. For example, ^{68}Ga-DOTATATE (^{68}Ga-DOTA-(Tyr^3)-octreotate) PET/CT has shown superiority in neuroendocrine tumor diagnosis than some currently FDA approved imaging methods [148]. ^{123}I-MIBG is used as a standard functional imaging for initial evaluation of neuroendocrine tumors. ^{68}Ga-DOTATATE is superior to ^{123}I-MIBG in detecting lesions in all anatomical locations, especially bony lesions. In one study of 15 recruited patients, the lesions in 4 patients were picked up by ^{68}Ga-DOTATATE but missed by ^{123}I-MIBG [149]. Schmid-Tannwald et al. proved that the detection rate of pancreatic NET with ^{68}Ga-DOTATATE PET/CT was significantly higher than that with MRI. The NETs were detected in 8/23 (34.8%) and 9/23 (39.1%) patients on T_2w imaging by observers but with 100% on PET/CT [150]. Goel et al. found that ^{68}Ga-DOTATATE PET detected bone metastases at a higher rate than CT: out of a total of 225 lesions detected by ^{68}Ga-DOTATATE PET, only 84 lesions could be detected by CT scans [151]. Besides, compared with well-established clinical PET imaging agent, ^{18}F-FDG and ^{68}Ga-DOTATATE could provide additional tumor information [152–154]. When NETs are in the initial stage, they have a slow metabolic activity and a high SSTR2 expression, resulting in a low uptake of ^{18}F-FDG but high uptake of ^{68}Ga-DOTATATE. When the tumor characteristics change into poorly differentiated ones, they have high ^{18}F-FDG uptake but low ^{68}Ga-DOTATATE uptake. In a study of 38 patients with primary or recurrent NET [153], the standard uptake value of ^{68}Ga-DOTATATE and ^{18}F-FDG was 29 vs. 2.9 in low-grade NET but 4.4 vs. 11.7 in high-grade NET (Fig. 6). It is generally believed that ^{18}F-FDG PET reflects the glucose metabolism and is more sensitive for tumor staging, while radiolabeled peptides target the receptor and have more potential value for assessing tumor grade and selection of patients suitable for receptor-targeted therapies.

6. Conclusion

The development of peptide based imaging agents relies on the cooperative efforts from biologists to discover new disease-related targets and corresponding targeting peptides, chemists to synthesize and characterize the peptide-based probes, engineers and medical physicists to improve imaging quality, as well as clinicians to assess the clinical value.

In recent years, numerous peptides have been discovered and designed for molecular imaging, including PET, SPECT, MRI, optical imaging, photoacoustic imaging and Raman imaging. Among these, radiolabeled peptides for PET and SPECT imaging are considered as the best candidates for clinical translation. Optimization of radiolabeled peptides can be achieved via chemical modification. First, cyclization of peptides can help restrict their three-dimensional conformation and improve receptor binding specificity and stability. Second, incorporation of an appropriate functional group such as glycosylation, PEGylation and human albumin may result in improved pharmacokinetic properties. Third, multimerization of targeting peptides (homo or hetero) can enhance the binding affinity due to the multivalency effect. Fourth, site-specific labeling or insertion of an appropriate linker between targeting peptides and radioisotopes helps retain the binding affinity and imaging activity. Fifth, a simple, automatic, high-yield labeling method is significant for clinical use. It is also worth to mention that development of multifunctional imaging probes, especially nanomaterial based imaging probes, which combines the advantages of different imaging modalities is of great interest, yet the pharmacokinetics and toxicities need careful investigation before possible use in the clinic. One typical example is the clinical trial of Bradbury group's ^{124}I-cRGDY-PEG-C dots [155].

Many radiolabeled peptides have been introduced into the clinic with impressive sensitivity and accuracy for tumor assessment. However, only several have been approved by FDA till now. One major challenge is how to obtain a significant foothold in the clinic, especially compared with well-established clinical imaging agents such as ^{18}F-FDG. Efforts are still needed in developing more sensitive, specific, and stable peptide-based diagnostics without unwanted tissue uptake and possible side effect. The clinical potential of existing probes on tumor grading also needs to be evaluated with more comprehensive information based on large scale clinical trials. What's more, the development of radionuclide based peptides can greatly benefit individualized theranostics, where imaging and the radiotherapy are carried out using the same vector by exchanging the imaging and therapeutic radionuclides. Quantifying the receptor density and distribution via PET/SPECT imaging would help design the therapeutics based on the same ligand and the same target. It is anticipated that these efforts can assist personalized therapy including early detection, staging, therapy planning and monitoring.

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Abbreviation

BBN	bombesin
CCK	cholecystokinin
CXCR4	chemokine receptor type 4
DADT	diaminedithiols

Delt-II	Deltorphin-II
DOTA	1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid
DTPA	diethylenetriaminepentaacetic acid
EB	Evans blue
EDC	carbodiimide
FDA	Food and Drug Administration
FDG	fludeoxyglucose
^{18}F-FB	^{18}F-fluorobenzoyl
FRET	fluorescence resonance energy transfer
^{18}FSFB	*N*-succinimidyl-4-^{18}F-fluorobenzoate
^{68}Ga-DOTATATE	^{68}Ga-DOTA-(Tyr^{3})-octreotate
GBM	glioblastoma multiforme
GLP-1	glucagon-like peptide 1
GRP	gastrin-releasing peptide
GRPR	gastrin-releasing peptide receptor
HYNIC	2-hydrazidonicotinic acid
MAG3	mercaptoacetylglycilgylcilgylicine
MC-1r	melanocortin-1 receptors
MIBG	metaiodobenzylguanidine
MMPs	metalloproteinases
MRI	magnetic resonance imaging
α-MSH	α-melanocyte-stimulating hormone
NET	neuroendocrine tumor
NHS	*N*-hydroxysuccinimidyl
NK_1R	neurokinin 1 receptor
NIRF	near infrared fluorescence
NOTA	1,4,7-triazacyclononane-1,4,7-triacetic acid
NT	neutrotensin
PEG	polyethyleneglycol

PET	positron emission tomography
PRTR	peptide receptor targeted radiotherapy
QDs	quantum dots
RGD	Arg-Gly-Asp
SIB	*N*-succinimidyl-5-iodo-3-pyridinecarboxylate
SIPC	*N*-succinimidyl-3-iodobenzoate
SPECT	single photon emission computed tomography
SRS	somatostatin receptor scintigraphy
SST	somatostatin
SWNTs	single-walled carbon nanotubes
TETA	1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid
TMTP1	tumor molecular targeted peptide 1
VIP	vasoactive intestinal peptide

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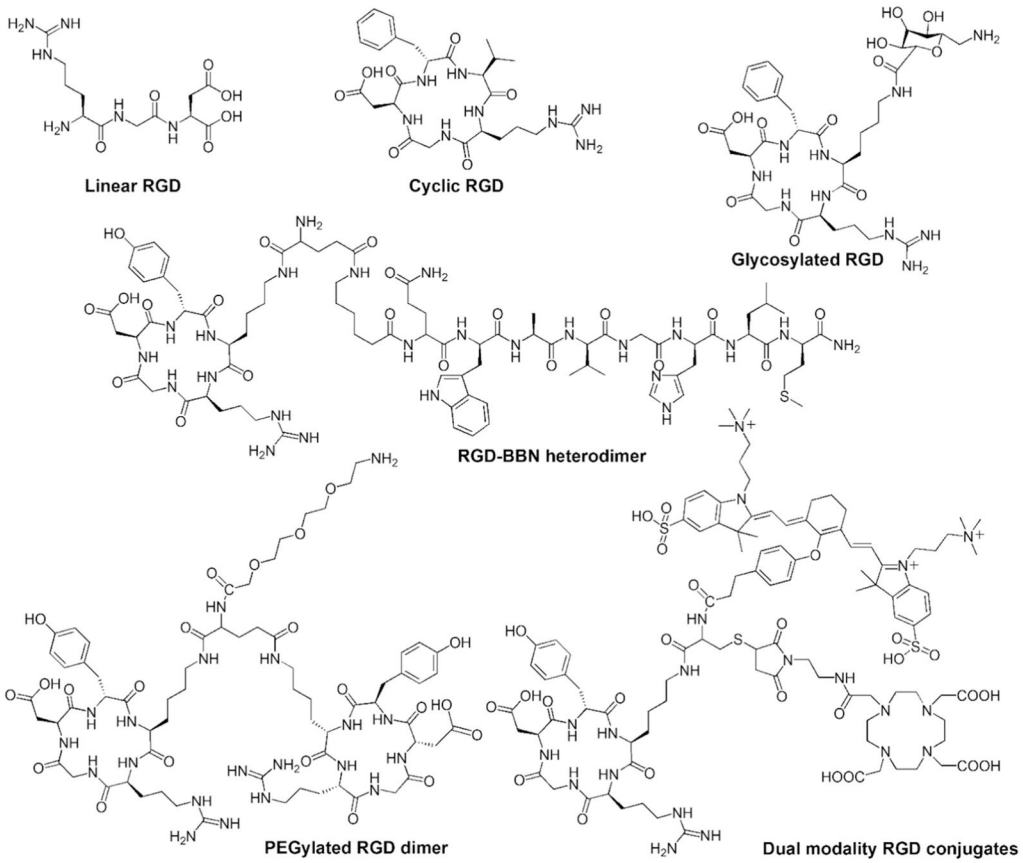

Fig. 1.

Examples of chemical modification of RGD peptides. Cyclization of RGD sequence increases the biostability and binding selectivity for integrin (modified from reference [97]). Glycosylation (modified from reference [82]) and PEGylation (modified from reference [88]) improve the blood half-life of RGD. Formation of homodimeric (modified from reference [88]) or heterodimeric (modified from reference [98]) peptide enhances the receptor targeting ability. Introduction of hybrid derivatives containing both a fluorescent and radioactive label allows the dual modality imaging (modified from reference [83]).

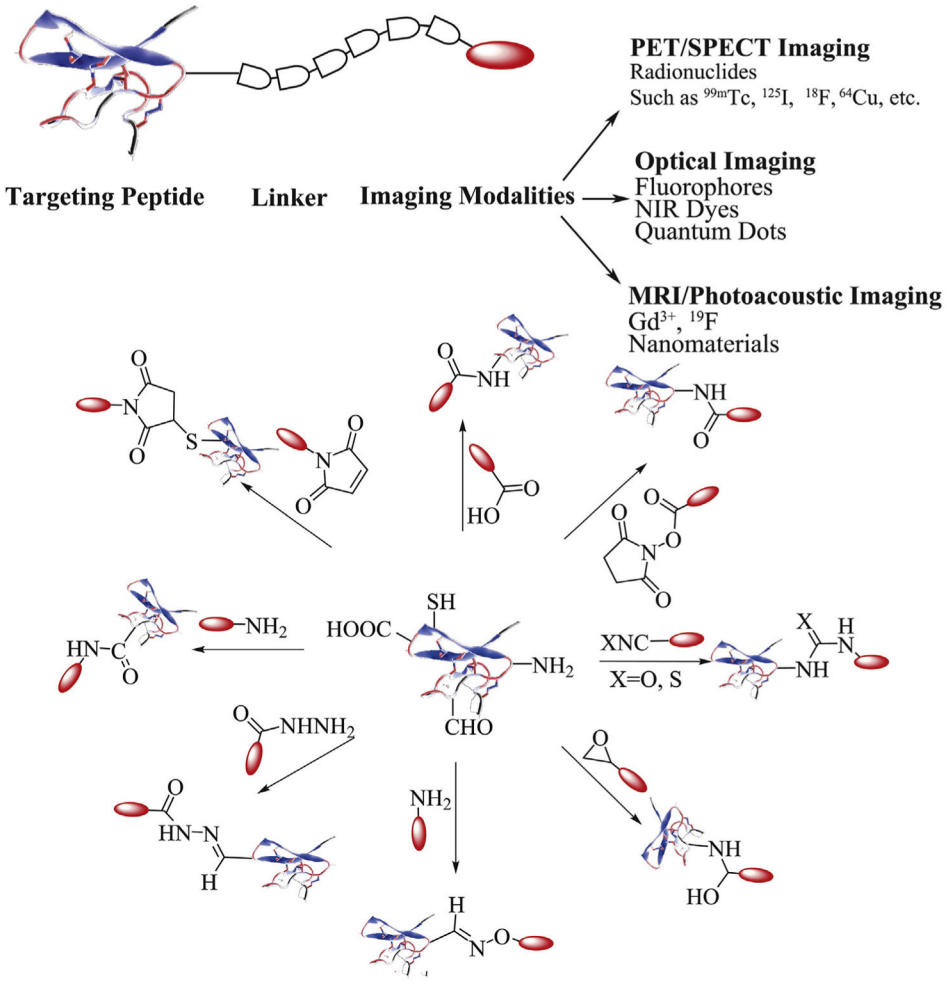

Fig. 2.

A general schematic diagram of functionalization chemistries of peptide based imaging probes (modified from reference [11] and reference [100]).

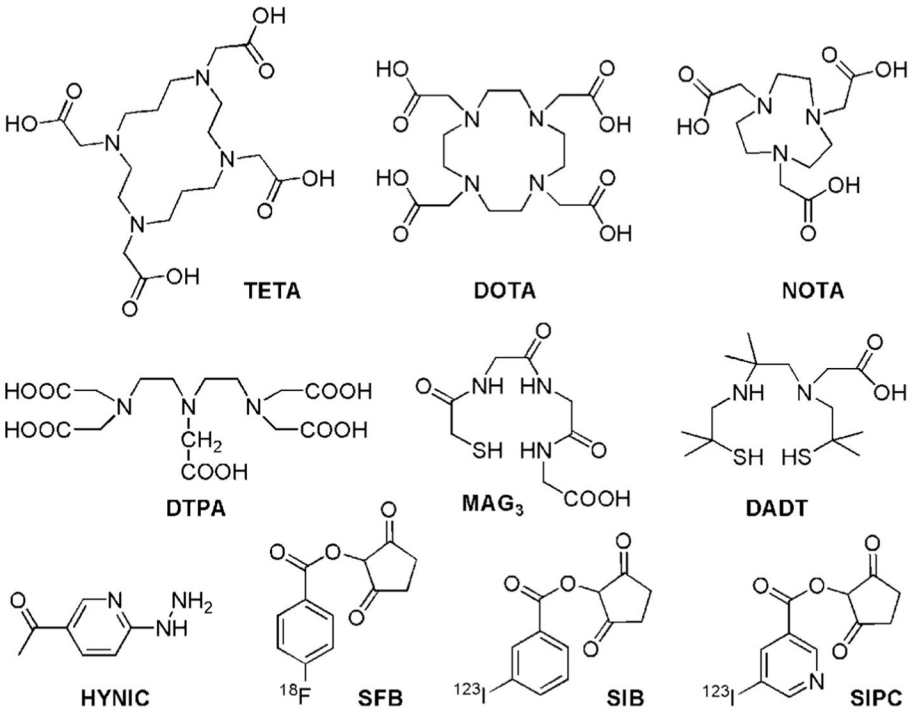

Fig. 3.

Selected labeling chelators and post-synthetic groups: 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid (TETA), 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA), diethylenetriaminepentaacetic acid (DTPA), mercaptoacetylglycilgylcilgylicine (MAG_3), diaminedithiols (DADT), 2-hydrazidonicotinic acid (HYNIC), *N*-succinimidyl-4-^{18}F-fluorobenzoate (^{18}FSFB), *N*-succinimidyl-5-iodo-3-pyridinecarboxylate (SIB) and *N*-succinimidyl-3-iodobenzoate (SIPC).

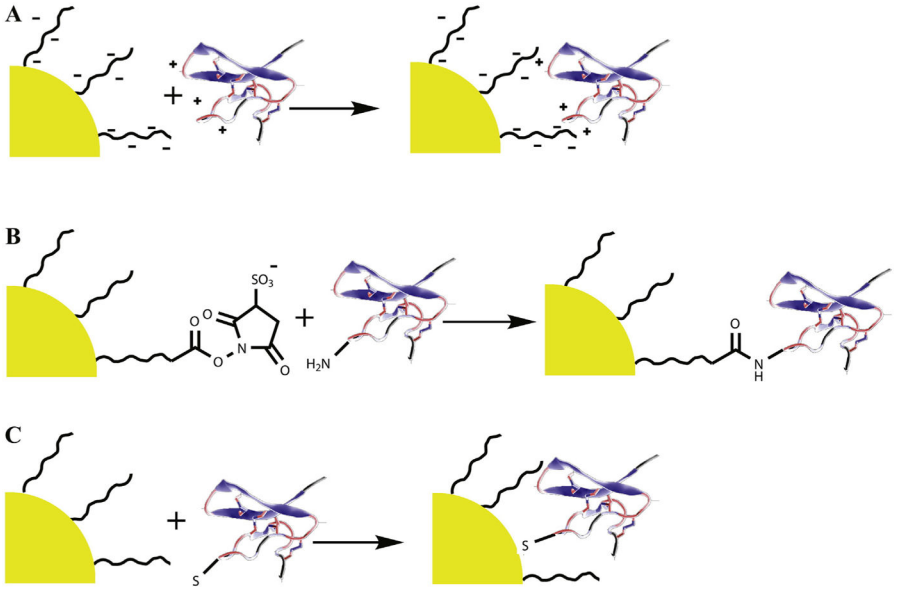

Fig. 4.

Three general schemes used for the construction of peptide-nanocomplex: (A) electrostatic interaction between opposite charges on the NP surface and the peptide; (B) covalent chemical attachment using classical bioconjugation chemistry like amine-carboxyl coupling; and (C) direct interaction between certain peptide motif and NP surface with high affinity. (modified from [111]).

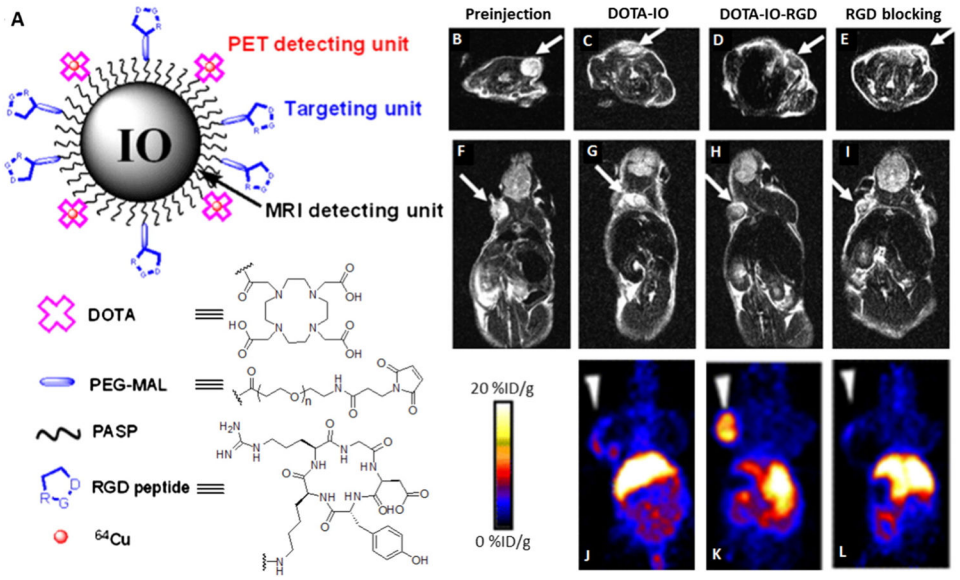

Fig. 5.

(A) A representative peptide-targeting MRI/PET dual modality nanoprobe. (B–I) T_2-weighted MR images and (J–L) decay-corrected whole-body coronal PET images of nude mice bearing U87MG tumor before (B, F) and 4 h after injection of ^{64}Cu-DOTA-iron oxide (IO) nanoparticles (C, G, J) ^{64}Cu-DOTA-IO-RGD (D, H, K) and ^{64}Cu-DOTA-IO-RGD with a blocking of RGD (E, I, L) [116].

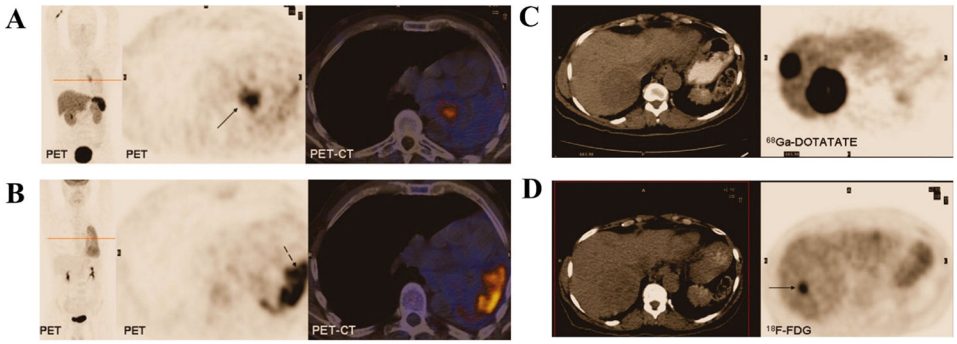

Fig. 6.

(A) ^{68}Ga-DOTATATE and (B) ^{18}F-FDG PET/CT images from a patient with well-differentiated bronchial carcinoid tumor. Histology showed that the left lung had postcollapse pneumonitis that was negative for ^{68}Ga-DOTATATE but positive for ^{18}F-FDG. (C) ^{68}Ga-DOTATATE and (D) ^{18}F-FDG PET/CT images in a 54-year-old female patient with metastatic carcinoid tumor. The lesions show ^{68}Ga-DOTATATE uptake along the whole circumference of the lesion, whereas there is only focal ^{18}F-FDG uptake at the margin [153].

Table 1

Some representative peptides, receptors and corresponding imaging applications.

Peptide designation	Key sequence	Receptor	Indication	Subject studied	Reference
RGD	Arg-Gly-Asp	Integrin	Angiogenesis	In vitro Rodents Humans	[28–32]
Bombesin/gastrin-releasing peptide	Share sequence of Trp-Ala-Val-Gly-His-Leu-Met	GRPR	Prostate cancer; breast cancer; glioma	In vitro Rodents Humans	[33–36]
CCK/gastrin	Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe	CCK2R	Medullary thyroid cancer	In vitro Rodents	[37–39]
VIP	His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-Arg- Lys-Gln-Met-Ala-Val-Lys-Lys-Tyr-Leu-Asn-Ser-Ile-Leu-Asn	VPAC1/2	Primary and metastatic lesions of breast, ovarian, prostate, colon and urinary bladder carcinomas, and meningiomas	In vitro Rodents Humans	[13,40,41]
NT	pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu	NTSR1	Tumor progression (lung cancer/breast cancer/prostate cancer)	In vitro Rodents	[42,43]
Somatostatin	Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys (Disulfide bridge Cys^3-Cys^{14})	SSTR1–5	Neuroendocrine tumors	In vitro Rodents Humans	[14,44–46]
α-MSH	Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val	Melanocortin-1 receptor	Melanogenesis	In vitro Rodents	[47,48]
T140	Arg-Arg-Nal-Cys-Tyr-Arg-Lys-D-Lys-Pro-Tyr-Arg-Cit-Cys-Arg	CXCR4	Tumor aggressiveness, invasiveness, and metastasis formation	In vitro Rodents	[49,50]
Exendin-4	His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met- Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn- Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser	GLP-1R	Insulinomas	In vitro Rodents Humans	[51–53]
NPY	Tyr-Pro-Ser-Lys-Pro-Asp-Asn-Pro-Gly-Glu-Asp-Ala-Pro-Ala- Glu-Asp-Leu-Ala-Arg-Tyr-Tyr-Ser-Ala-Leu-Arg-His-Tyr-Ile- Asn-Leu-Ile-Thr-Arg-Gln-Arg-Tyr	NPY1R	Breast cancer; sarcomas	In vitro Rodents	[54,55]
Substance P	Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met	NK1R	Glioblastoma	In vitro Rodents	[56,57]
TMTP1	Asn-Val-Val-Arg-Gln	Unclear	Highly metastatic cancer cells	In vitro Rodents	[58,59]